# A COMPARISON OF TWO NONINVASIVE TECHNIQUES TO MEASURE TOTAL BODY LIPID IN LIVE BIRDS

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ABSTRACT.—Total body electrical conductivity (TOBEC) and near infrared interactance (IRI) are noninvasive techniques with potential utility for monitoring body composition of free-ranging birds or captive experimental subjects. I used live Northern Bobwhites (*Colinus virginianus*) to validate these methods for estimation of total body lipid. Total body electrical conductivity and IRI measurements were compared with body composition as determined by solvent extraction of carcasses.

I measured IRI at three body sites (subscapular, pectoralis, and subfemoral) on 42 bobwhites. Only the subscapular and pectoralis sites yielded IRI measurements that were significantly correlated with percent body lipid, but IRI from these sites explained only 17.5% and 10.0% of the variation in percent body lipid, respectively. The cause of the low correlations is unknown, but it was not a function of differences in subject posture or light-wand position between replicate measures on the same subject.

Total body electrical conductivity measurements explained 92% of the variation in lean body mass in a sample of 62 bobwhites. Measurements were affected by small differences in subject movement and position in the measurement chamber. There was no significant difference in TOBEC of subjects with or without aluminum leg bands. Measurements obtained from the same subjects with two different SA-1 TOBEC analyzers were not significantly different. The residuals about the regression of TOBEC against lean body mass were not correlated significantly with body temperature, but were significantly correlated with hydration. Total body electrical conductivity explained 99.1% of the variation in lean body mass when only those data from normally hydrated and properly restrained subjects were included ($n = 38$). Estimated body lipid from TOBEC measurements was highly correlated with total body lipid ($R = 0.96$) as determined by solvent extraction. The 95% confidence limits from the inverse regression procedure for the estimate of total body lipid from TOBEC measurements were ±4.75 g at 25 g lipid, ±4.85 g at 10 g lipid, and ±5.62 g at 50 g lipid. Estimated total body lipid from TOBEC measurements was on average within 10% of total body lipid from solvent extraction. Although IRI analysis failed to yield a suitable index to body composition, TOBEC analysis provided an accurate estimate of total body lipid in live bobwhites.
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BIRDS are highly active, obligate homeotherms that maintain high body temperatures (38–42°C) and have few options for reducing energy expenditure rates. Survival during periods of negative energy balance, such as winter or migration (Blem 1980), is related to the quantity of fat stored during periods of energy surplus. In some species, reproductive success is dependent on the parents' ability to recover from the rigors of winter and migration and to deposit sufficient energy reserves to meet demands for courtship, egg production, incubation, and brood-rearing (King 1973). Energy is an important currency of fitness for birds, and lipid depots are the primary form of energy reserves (Drent and Daan 1980, Blem 1990).

Measurements of body composition have been used to evaluate the energetic cost of growth, reproduction, molt, migration, and other components of avian life histories (reviewed in Ricklefs 1974, Walsberg 1983, Blem 1990). Seasonal patterns of lipid deposition and catabolism identify those periods when energy is limiting and how foraging behavior is adjusted to meet these constraints (Blem 1976, Drobney 1980). Such studies have provided considerable insight into the factors responsible for the evolution of avian life-history traits.

Considerable information on the nutritional requirements of birds and the impact of environmental contaminants has been gained through controlled experiments with captive stock. In these studies, a sample of subjects is maintained on a particular dietary regime for a prescribed period, after which the subjects are sacrificed and body composition is analyzed

(Scott 1973). Periodic weighings have been used to nondestructively track body composition over the course of an experiment, but total body fat may be poorly correlated with total body mass (Blem 1990: 71).

Proximate analysis to determine total body lipid (percent of total body mass) is a fundamental method for evaluating physical condition and nutritional status of animals (Dobush et al. 1985). This method is time-consuming and expensive, and it requires killing the subject for analysis. Despite the importance of body-composition data as an index to physiological condition, destructive methods are not feasible where endangered species or valuable zoo specimens are concerned. Even in the case of larger, stable populations, there is growing public concern over the ethics of sacrificing a sufficiently large sample of individuals to draw statistically valid conclusions. In addition, destructive sampling precludes repeated measurement of the same individual to ascertain temporal variation in fat depots.

Over the last 15 yr, ornithologists have sought to devise nondestructive methods to determine physical condition and energy reserves of birds. Both invasive and noninvasive techniques have been investigated, including blood chemistry (LeMaho et al. 1981), labeled water dilution space (Nagy and Costa 1980), fat scoring (Krementz and Pendleton 1990), body mass adjusted for morphological measurements (Johnson et al. 1985), and ultrasonic techniques (Baldassarre et al. 1980). All of these indirect techniques, however, have limited ability to monitor precisely temporal variation in lipid reserves.

In the last few years, two new noninvasive techniques with the potential to measure accurately total body lipid have attracted attention in the field of human clinical nutrition (reviewed in Lukaski 1987). The total body electrical conductivity (TOBEC) method was first developed for use in analyzing the chemical composition of foodstuffs, specifically ground meat and live hogs (Harker 1973, Domermuth et al. 1976). Subsequently, the method was modified for clinical studies of human body composition (Harrison and Van Itallie 1982; Presta et al. 1983a, b; Harrison 1987, Van Loan et al. 1987). The TOBEC method relies on the major difference in conductivity between lipids and other body constituents to estimate total lean body mass (Van Loan and Mayclin 1987). The much higher conductivity of lean body constituents is a function of their higher water and electrolyte content (Pethig 1979). The difference between total body mass determined by weighing and lean body mass estimated by TOBEC provides an estimate of total body lipid. Subjects are placed in a chamber surrounded by a solenoid, which alters the electromagnetic inductance of the coil. The change in inductance is determined primarily by the subject's electrical conductivity. A major advantage of the technique is that measurements can be obtained rapidly and easily. Validation studies to date indicate that accuracy of TOBEC estimates of lean body mass can be high if care is taken to insure that subjects are (1) properly positioned in the chamber, (2) not hyperthermic, and (3) normally hydrated (Bracco et al. 1983, Walsberg 1988, Keim et al. 1988, Castro et al. 1990).

Near infrared interactance (IRI) analysis uses low-energy electromagnetic radiation in the wavelength range of 600–2,500 nm (Rosenthal 1986). The method is based on the principle that differences in the physical character of hydrogen bonds between water, protein, carbohydrates, and lipids result in differences in near infrared absorption that are correlated with body composition (Futrex, Inc., 1988). The IRI technique was initially developed by the U.S. Department of Agriculture in 1965 to measure the composition of foodstuffs. The technology has been used widely to control quality in the grain industry (Rosenthal 1977; Norris 1983a, b, 1985), to measure fat content of raw meats (Lanza 1983), and to measure human body composition, particularly percent body fat (Conway et al. 1984, Conway and Norris 1987). In these latter studies, IRI data from a series of body sites were compared by linear regression with independent estimates of body fat obtained by standard clinical techniques. The $r^2$ of the regression of results from IRI analysis on deuterium dilution-predicted fat was 0.83, with a SEE of 3.1% (Conway and Norris 1987). Conway and Norris (1987) concluded that IRI analysis had potential as a field method for measuring body composition, but further validation was necessary before the technique could be widely applied. Regressed against a direct measure of body composition, such as solvent extraction, IRI data would reliably assess the accuracy of the technique.

Near infrared interactance fat analyzers have recently been developed that are compact and considerably more portable than instruments required for TOBEC analysis. The Futrex-5000

computerized spectrophotometer is a small, inexpensive, battery-powered IRI instrument with the potential to estimate body composition over a wide range of subject body sizes. This instrument uses a near infrared emitting and detecting "wand" placed on the skin surface during measurements. Consequently, subjects large enough for placement of the light wand on the body surface can be measured. The instrument calculates interactance at two different wavelengths by dividing the signal obtained at the body site by the signal from a reflectance standard (1-cm thick Teflon block). The radiation intensity emitted by the light wand equals a 60-watt light bulb at 6 m, penetrates ca. 1 cm into tissues, and is considered safe for live subjects (Futrex, Inc., 1988).

I attempted to validate the IRI technique on live avian subjects. Walsberg (1988) and Castro et al. (1990) previously validated the TOBEC technique to estimate lean body mass in a variety of avian species, but neither determined the accuracy of the TOBEC technique to estimate total body lipid within a single species. This is crucial if the technique is to be used to track changes in body composition of individual subjects over time, a major advantage of nondestructive techniques.

## Methods and Materials

*Subjects.*—Northern Bobwhites (*Colinus virginianus*) were chosen for study because they are small enough to fit into a commercially available TOBEC apparatus and large enough to permit IRI measurement at several different body sites. I purchased 72 bobwhites from a local breeder in early October 1989. Individuals were assigned randomly to six groups of 12, and each group was housed in separate, raised outdoor pens (4.9 × 1.2 × 1.2 m high). Two-thirds of each pen was covered, and windbreaks were on 3 sides. Each pen had a water container and feed tray at each end and evergreen boughs for cover. Fresh water and food were provided *ad libitum* on a daily basis. Two pens of subjects (24 individuals) were placed on each of 3 different dietary regimes. One group received a high protein (minimum 24.0%) game-bird starter feed (Siemer Gamebird Starter-Grower). The second group received a lower protein (13.0%) game-bird maintenance feed (Siemer Gamebird Maintenance). The third group received a high-energy diet of a 2:1 mixture (v:v) of medium cracked corn (ca. 8% protein) and game-bird maintenance feed. The three dietary regimes were designed to produce subjects with a range of body lipid levels, but metabolizable energy content of each diet was not measured. Subjects were maintained on their respective diets for a minimum of 12 weeks (early October to early January) before the initiation of TOBEC and IRI measurements. Most subjects were measured in midwinter, when bobwhites normally have maximal body fat reserves (Robel 1972).

In addition, 11 wild bobwhites were live-trapped at Crab Orchard National Wildlife Refuge in mid-November and kept in a separate pen under the same conditions described above. Wild bobwhites were fed the high-energy diet described above for at least 12 weeks before measurement of body composition.

*Total body electrical conductivity.*—Preliminary trials indicated that ambient temperature, wind, and lighting conditions had a significant effect on both TOBEC and IRI measurements. Consequently, subjects were moved indoors for measurements. Each subject was restrained in a nylon stocking and weighed on a Fisher top-loading balance (±0.01 g). We used a protocol similar to that described by Walsberg (1988) to measure total body electrical conductivity with the SA-1 Small Animal Body Composition Analyzer (EM-SCAN, Inc., Springfield, Illinois). Before measurement, the calibration of the instrument was checked by reading (1) the reference number, (2) the data number with the sample chamber empty, and (3) the data number with the calibration tube inserted in the sample chamber. Subject measurements were taken by (1) reading the reference number, (2) inserting the empty sample tube into the sample chamber and reading the resulting data number, (3) inserting the sample tube with animal into the sample chamber and reading the resulting data number, (4) reiterating steps 2 and 3 six times, and (5) reading the reference number again. An index of lean body mass was calculated as

$$\text{EM-SCAN number} = [(N_1 - N_2)/R]C,$$

where EM-SCAN number is an index of lean body mass, $N_1$ is the average data value for the six iterations when the animal was not in the chamber, $N_2$ is the average data value for the six iterations when the animal was in the chamber, $R$ is the average of the two reference numbers, and $C$ is a normalization constant specific to each instrument. This protocol was designed to minimize error associated with variation in the position of the subject in the chamber. Immediately following completion of the TOBEC measurements, I measured deep proventricular body temperature with a BAT-12 thermocouple thermometer (±0.1°C).

After TOBEC analysis, an aluminum leg band was attached to 29 of the subjects and the TOBEC protocol was repeated. In another subset of subjects ($n$ = 12), the TOBEC protocol was repeated with a second SA-1 Analyzer (identical model) to determine consistency of measurements between instruments.

*Near infrared interactance.*—After completion of the TOBEC protocol, I used the Futrex-5000 (Futrex, Inc., Gaithersburg, Maryland) computerized spectrophotometer to measure near infrared interactance (IRI).

TABLE 1. Morphometrics of Northern Bobwhites used in validations of the total body electrical conductivity and near infrared interactance nondestructive body composition analysis techniques.

| Parameter | $\bar{x} \pm$ SD | Range | $n$ |
|---|---|---|---|
| Total body mass (g) | 228.3 ± 25.0 | 171.8–276.9 | 62 |
| Game farm-reared | 237.7 ± 15.8 | 206.8–276.9 | 51 |
| Wild-caught | 185.2 ± 8.3 | 171.8–197.5 | 11 |
| Total body lipid (% live mass) | 9.5 ± 2.7 | 4.9–19.2 | 62 |
| Game farm-reared | 9.7 ± 2.9 | 4.6–19.2 | 51 |
| High-energy diet | 10.6 ± 3.1 | 5.9–17.6 | 14 |
| Intermediate diet | 9.7 ± 3.4 | 4.6–19.2 | 16 |
| Low-energy diet | 9.1 ± 2.3 | 5.8–14.2 | 21 |
| Males | 10.8 ± 3.0 | 5.9–19.2 | 27 |
| Females | 8.4 ± 2.3 | 4.6–14.6 | 24 |
| Wild-caught | 8.9 ± 1.7 | 7.1–11.9 | 11 |
| Males | 9.3 ± 1.9 | 7.5–11.9 | 5 |
| Females | 8.6 ± 1.6 | 7.1–10.5 | 6 |
| Total body lipid (g) | 21.6 ± 7.5 | 10.4–50.2 | 62 |
| Game farm-reared | 22.8 ± 7.7 | 11.3–50.2 | 51 |
| Wild-caught | 16.4 ± 3.7 | 10.4–22.6 | 11 |

In a room darkened to minimize interference from ambient light, I measured IRI at three different anatomical locations on each subject (subscapular, pectoralis, and subfemoral). Before measurement, each site was plucked to provide bare skin for placement of the light wand. The three sites were chosen partly because they coincide with apterylae, which minimized the need for extensive plucking of contour feathers. Before IRI measurements, an outline of the light wand was drawn on the skin at each site with a felt-tipped pen to insure that replicates were taken at the same site. I measured by firmly pressing the end of the light wand against the subject's skin within the drawn outline. I scanned each site 4 times in a series of 6 replicates, for a total of 24 scans per site. Before taking each replicate set of measurements, I inserted the probe into a Teflon optical standard and took 4 reference measurements at each wavelength. Considerable care was taken to maintain consistent subject posture and light-wand placement between replicates and subjects.

The Futrex-5000 can be programmed to output "optical density" (OD) values at 940 and 950 nm wavelengths. Optical density values are equivalent to log 1/interactance that vary linearly with body composition. These values were standardized by taking the difference between the average of the reference measurements and the baseline reference (measured at the beginning of the study) at the respective wavelengths, and subtracting this difference from the average subject measurement. This yielded a corrected OD value at 940 nm and at 950 nm for each body site. In clinical nutrition applications, both the corrected OD values and the difference between corrected OD values at 940 and 950 nm are used to predict percent body fat (Futrex, Inc., 1988). Consequently, I calculated the difference between the corrected OD values at the two wavelengths for each body site. Immediately after the IRI protocol, deep proventricular temperature was measured and subjects were quickly and humanely sacrificed by thoracic compression. Carcasses were weighed to the nearest 0.01 g, placed in double plastic bags and frozen at −20°C.

*Carcass analysis.*—Carcasses were partially thawed, weighed, plucked, and reweighed to determine feather mass by subtraction. Crop contents were then removed and weighed to the nearest 0.01 g. Plucked whole carcasses were air-dried at 60°C in a convection oven to constant mass. Dried carcasses were weighed to determine water content by subtraction, then ground and homogenized by passing them 6 times through a meat grinder.

Extraction thimbles were dried at 60°C for at least 24 h, weighed to the nearest 0.1 mg on a Sartorious analytical balance, and loaded with 2–3 g of dry infusorial earth (Fisher Scientific) to prevent clogging of thimble pores during extraction. Three aliquots (2–3 g) of the dried carcass homogenate were weighed (±0.1 mg) into tared extraction thimbles with infusorial earth. Loaded thimbles were placed in a convection oven at 60°C overnight and reweighed before extraction. I used a Soxtec System HT6 (Tecator, Inc., Herndon, Virginia) solvent extraction apparatus and 5:1 (v:v) petroleum ether and chloroform to extract total lipids from the three aliquots. Extracted lipids were weighed to determine lipid content (% dry mass) for each aliquot. The lipid content of each dried carcass was determined from the mean of the three aliquots. If the standard deviation of the mean exceeded 1%, additional aliquots were extracted until the SD was less than 1% (averaged SD = 0.255%, $n$ = 62). Total body lipid (g) was calculated from the product of lipid content (% dry mass) and dry carcass mass. Lean body mass was calculated as the difference be-

tween total body mass and total body lipid mass. Lipid content (% live mass) was calculated using the following formula:

$$\text{Lipid content (\%)} = TBL/(FM_B - CC) \times 100, \quad (1)$$

where $TBL$ is total body lipid, $FM_B$ is fresh body mass immediately after death, and $CC$ is the mass of crop contents.

*Data analysis.*—To explore the relationship between IRI at the three body sites and percent body lipid as determined by solvent extraction, I used least squares linear and multiple regression analysis. I calculated regressions of each set of IRI measurements against total body lipid (% fresh body mass) both separately and together to determine the best predictor(s).

The SA-1 was validated by regressing EM-SCAN number against lean body mass as determined by solvent extraction. To investigate the effects of other variables (body temperature, total body water, total body lipid, total feather mass, date of analysis, sex) on EM-SCAN number, I regressed the residuals about the regression of EM-SCAN number on lean body mass against each variable. I used the EM-SCAN number for each subject to calculate TOBEC-estimated total body lipid, and the least squares regression equation to estimate lean body mass. Estimated lean body mass was subtracted from live body mass to provide an estimate of total body lipid. TOBEC-estimated total body lipid was then regressed against total body lipid as determined by solvent extraction. Inverse regression (Sokal and Rohlf 1981) was used to calculate confidence limits for TOBEC-estimated total body lipid at different levels of total body lipid. Statistics were calculated using StatWorks 1.0.

## Results

*Carcass composition.*—A total of 62 bobwhites (51 game farm-reared, 11 wild-caught) were analyzed by the TOBEC technique (Table 1). Of the 51 game farm-reared quail, 42 quail were analyzed by IRI spectroscopy (20 males, 22 females). Average body mass of game farm-reared bobwhites (237.7 g) was significantly greater than that of wild-caught bobwhites (185.2 g, $t = 15.681$, $P < 0.001$); there was no overlap in total body mass between the two groups. However, total body lipid as a percent of fresh mass was not significantly greater in game farm-reared bobwhites compared with wild-caught bobwhites ($t = 1.1902$, $P > 0.05$, Table 1). Percent body lipid was significantly correlated with total body mass in both game farm-reared ($R = 0.371$, $P = 0.007$, $n = 51$) and wild-caught ($R = 0.675$, $P = 0.022$, $n = 11$) bobwhites, but total body mass explained only 14% and 46% of the variation in percent body lipid, respectively (Fig. 1).

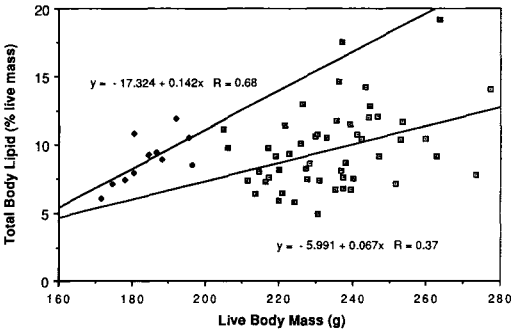


Fig. 1. Percent body lipid as a function of total live body mass in game farm-reared and wild-caught Northern Bobwhites. Dotted squares represent game farm-reared individuals, and solid diamonds represent wild-caught individuals.

Percent body lipid was not significantly correlated with dietary regime among game farm-reared bobwhites ($R = 0.202$, $P = 0.151$, $n = 62$). Birds presumably compensated for lower energy diets by consuming greater quantities of food. Percent body lipid was significantly correlated with date of analysis ($R = 0.418$, $F = 12.72$, df = 1/60, $P < 0.001$), which indicates that average body lipid levels slowly declined during the validation period (January 2 to March 14).

Live mass of male game farm-reared bobwhites was not significantly greater than females ($t = 1.435$, $P > 0.05$). However, game farm-reared males had significantly higher percent body lipid than females ($t = 3.323$, $P < 0.05$, Table 1). The same trend was apparent in wild-caught bobwhites (Table 1), but small sample sizes precluded statistical significance ($t = 0.698$, $P > 0.05$).

*Near infrared interactance.*—The corrected values for optical density (OD) at either 940 nm or 950 nm were not significantly correlated with percent body lipid for any of the three body sites ($P > 0.10$). Optical density at 940 nm was highly correlated with OD at 950 nm for both the subscapular ($R = 0.959$, $P < 0.0005$, $n = 42$) and pectoralis sites ($R = 0.931$, $P < 0.0005$, $n = 42$). The coefficient of variation in OD associated with variation in position of the light wand on each subject averaged 1.00% for subscapular OD at 940 nm and 0.99% at 950 nm ($n = 42$ individuals); 0.80% at 940 nm and 0.82% at 950 nm for pectoralis OD ($n = 42$); and 1.95% at 940 nm

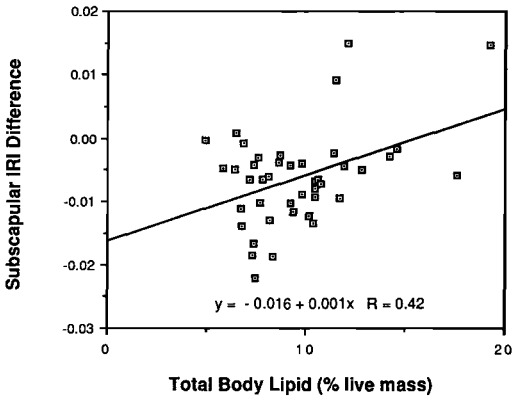


Fig. 2. Difference in optical density at 940 nm and 950 nm at the subscapular site as a function of percent body lipid in game farm-reared Northern Bobwhites.

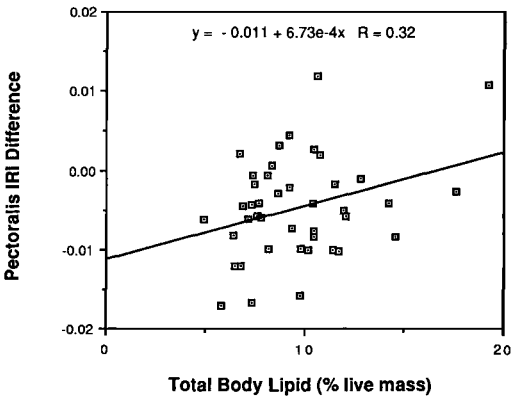


Fig. 3. Difference in optical density at 940 nm and 950 nm at the pectoralis site as a function of percent body lipid in game farm-reared Northern Bobwhites.

and 1.81% at 950 nm for subfemoral OD ($n$ = 29).

The difference between OD at 940 nm and 950 nm (OD difference) was correlated significantly with percent body lipid at the subscapular ($R$ = 0.42, $F$ = 8.452, df = 1/40, $P$ = 0.006, $n$ = 42; Fig. 2) and pectoralis sites ($R$ = 0.32, $F$ = 4.469, df = 1/40, $P$ = 0.038, $n$ = 42; Fig. 3). However, subscapular OD difference explained only 18% and pectoralis OD difference only 10% of the variation in percent body lipid. Optical density difference at the subscapular site was not significantly correlated with OD difference at the pectoralis site ($R$ = 0.090, $P$ = 0.579, $n$ = 42). Optical density difference at the subfemoral site was not significantly correlated with percent body lipid ($F$ = 0.038, df = 1/27, $P$ = 0.841).

When subscapular OD difference and pectoralis OD difference were included in a multiple regression model, these two independent variables explained only 25% of the variation in percent body lipid ($F$ = 6.612, df = 2/39, $P$ = 0.004, $n$ = 42). The multiple regression model that explained the greatest portion of the variance in percent body lipid included the variables subscapular OD difference, sex, and analysis date ($r^2$ = 0.571, $F$ = 16.838, df = 3/38, $P$ < 0.0005, $n$ = 42). No other independent variables explained a significant portion of the residual variation in percent body lipid when entered into the multiple regression model ($P$ > 0.05).

*Total body electrical conductivity.*—EM-SCAN number was significantly correlated with lean body mass ($R$ = 0.959) and EM-SCAN number explained 92% of the variation in lean body mass ($F$ = 695.1, df = 1/60, $P$ < 0.0005, $n$ = 62; Fig. 4). The coefficient of variation in EM-SCAN number associated with variation in position of the subject in the sample chamber averaged 1.243% (range = 0.209–4.216%, $n$ = 53 individuals). Estimated total body lipid from TOBEC measurements was significantly correlated with total body lipid, explaining 59% of the variation in total body lipid ($R$ = 0.768, $F$ = 86.06, df = 1/60, $P$ < 0.0005, $n$ = 62; Fig. 5).

There was no significant difference in EM-SCAN number for subjects with and without aluminum leg bands (paired $t$ = 0.470, $P$ = 0.647, $n$ = 29 pairs). There was also no significant difference in EM-SCAN number from the two different instruments (paired $t$ = −0.193, $P$ = 0.844, $n$ = 12 pairs).

The absolute values of the residuals about the regression of EM-SCAN number on lean body mass were negatively correlated with date of analysis ($R$ = 0.446, $F$ = 14.92, df = 1/60, $P$ = 0.001, $n$ = 62). A plot of the residuals vs. date of analysis indicated that the precision of TOBEC measurements increased over the validation period (Fig. 6). Specifically, for subjects analyzed before 23 January we cut a hole in the nylon stocking restraint so that the subject's head protruded from the stocking. After 23 January the subject's entire body, including its head, was enclosed in the stocking. When the data collected before 23 January were removed from the data set, there was no longer a significant correlation between the residuals and analysis date ($R$ = 0.127, $P$ = 0.576, $n$ = 43). Consequently, data from bobwhites analyzed before 23 January were removed from the data set.

In the new data set, EM-SCAN number ex-

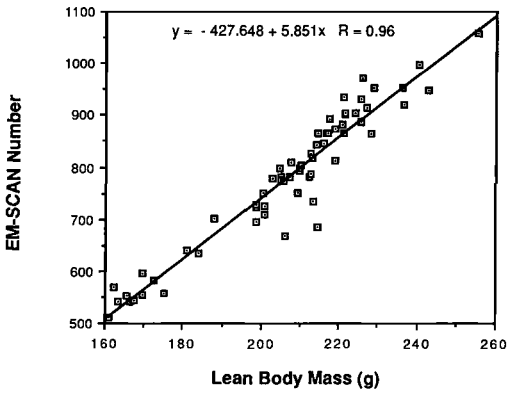


Fig. 4. EM-SCAN number (TOBEC) as a function of lean body mass as determined by solvent extraction in Northern Bobwhites.

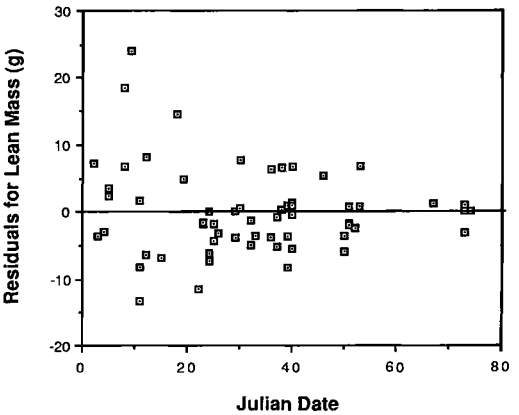


Fig. 6. Residuals about the regression of EM-SCAN number vs. lean body mass as a function of analysis date.

plained 98% of the variation in lean body mass ($r^2 = 0.980$, $F = 1976.2$, df = 1/41, $P < 0.0005$, $n = 43$), and estimated total body lipid from EM-SCAN number explained 83% of the variation in total body lipid ($r^2 = 0.832$, $F = 203.5$, df = 1/41, $P < 0.0005$, $n = 43$). The residuals about the new regression equation of EM-SCAN number vs. lean body mass were not significantly correlated with body temperature ($R = 0.044$, $F = 0.081$, df = 1/41, $P = 0.774$, $n = 43$). Average body temperature of subjects was 41.8°C ($n = 61$) and varied over a range of nearly 4°C (SD = 0.87, range = 39.7–43.6). The residuals were significantly correlated with total body water (% of lean mass, $R = 0.502$, $F = 13.14$, df = 1/39, $P = 0.001$, $n = 41$; Fig. 7). Five of six subjects whose body water was <66% of lean mass fell below the regression line of EM-SCAN number vs. lean body mass and included the four outliers in the model. When these data were removed, EM-SCAN number explained 99% of the variation in lean body mass ($r^2 = 0.991$, $F = 3965.2$, df = 1/36, $P < 0.0005$, $n = 38$). EM-SCAN number was related to lean mass as determined by solvent extraction by

$$\text{EM-SCAN number} = -413.93 + 5.831(\text{LM}), \quad (2)$$

where LM is lean body mass. The SE of the slope of the regression equation was ±0.093. Lean mass can be estimated from TOBEC measurements of bobwhites by the equation,

$$\text{LM} = (\text{EM-SCAN number} + 413.93)/5.831. \quad (3)$$

Estimated total body lipid from EM-SCAN number explained 92% of the variation in total body lipid ($r^2 = 0.917$, $F = 396.3$, df = 1/36, $P < 0.0005$, $n = 38$; Fig. 8). I used the inverse regression procedure to determine the 95% confidence limits for the estimate of total body lipid from TOBEC measurements: ±4.8 g at 25 g estimated body lipid, ±4.9 g at 10 g estimated body lipid, and ±5.6 g at 50 g estimated body lipid.

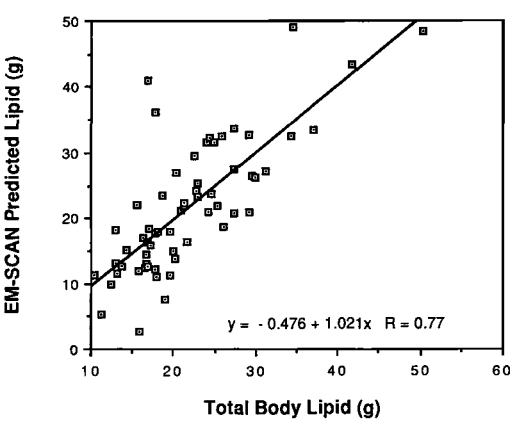


Fig. 5. Estimated total body lipid from EM-SCAN number as a function of total body lipid as determined by solvent extraction in Northern Bobwhites.

## Discussion

The near infrared interactance (IRI) results indicate serious limitations in the usefulness of the Futrex-5000 for estimation of body composition in bobwhites. The low correlation be-

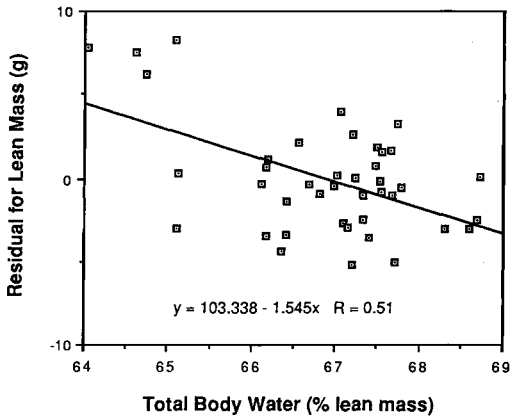


Fig. 7. Residuals about the regression of EM-SCAN number vs. lean body mass as a function of total body water (percent lean mass) in Northern Bobwhites.

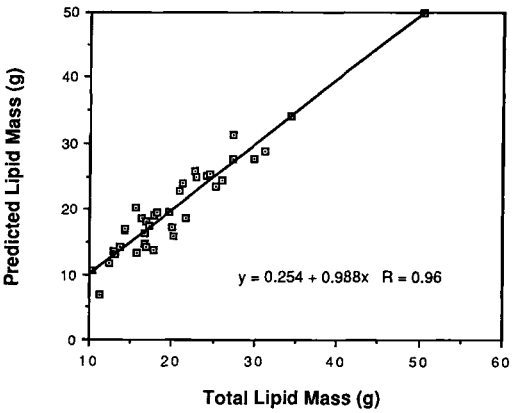


Fig. 8. Estimated total body lipid from EM-SCAN number as a function of total body lipid as determined by solvent extraction. Data collected from subjects analyzed before 23 January or from dehydrated subjects are not included.

tween IRI measurements and percent body lipid is unexplained. Except for subfemoral optical density measurements, the coefficients of variation of OD measurements for each subject were not higher than those for total body electrical conductivity measurements. Consequently, variation between replicate measures of OD due to variation in light-wand position, subject position, or pressure exerted on the light wand were apparently not responsible for the low correlation between OD and percent body lipid. Optical density measurements from different sites on the same subject were not significantly correlated. Consequently, either OD measurements failed to reflect differences in composition under the light wand, or these differences were weakly correlated with differences in total body composition. Differences between individuals, sexes, or both, in the distribution of fat depots may be related to preferential mobilization of lipid from certain depots.

The SA-1 Small Animal Body Composition Analyzer (TOBEC) is a promising method for tracking changes in body composition of experimental subjects. Normalized TOBEC measurements are apparently consistent among instruments and, as reported by Castro et al. (1990), aluminum leg bands do not significantly affect EM-SCAN number. Two factors affected the precision of lean mass estimates from EM-SCAN number. First, seemingly minor differences in subject posture within the sample chamber affected TOBEC measurements. This was demonstrated by the higher residuals about the regression of EM-SCAN number on lean mass when the subject's head was not included in the nylon stocking restraint. Second, care should be taken to avoid subject dehydration. In the present study, EM-SCAN number from dehydrated subjects underestimated lean mass. Walsberg (1988) obtained similar results with Gambel's Quail (*Callipepla gambelii*) deprived of water for 3 days. Alterations in the electrolyte concentration and composition of artificial "phantom" subjects are known to alter EM-SCAN number (Klish et al. 1984). Subjects with abnormally high extracellular fluid volume, however, do not significantly affect the accuracy of lean mass estimates from EM-SCAN number (Cochran et al. 1989). This is apparently due to the predominance of water in determining the conductivity of biological systems. In dehydrated subjects where body water constitutes a relatively small proportion of lean body mass, TOBEC measurements underestimate the true lean body mass. The lost precision associated with subject dehydration can be mitigated by efforts to minimize subject stress and the time interval between capture and measurement. However, TOBEC measurements from wild-caught birds whose hydration state is suspect (e.g. immediately following a long flight or under conditions of heat stress) should be treated with caution.

The results of the present TOBEC validation were compared with those of the only two pre-

viously published validations using live birds (Walsberg 1988, Castro et al. 1990). These studies used 11 and 8 species, respectively, to generate prediction equations for lean mass as a function of EM-SCAN number. Walsberg (1988) obtained an $r^2$ of 0.988 for a fitted second-order polynomial model, and Castro et al. (1990) obtained an $r^2$ of 0.95 for a linear model. These values are higher than the $r^2$ for the complete bobwhite data set reported in this study ($r^2$ = 0.920). Once data collected early in the present validation and those from dehydrated individuals were removed from the data set, however, the $r^2$ was 0.991. Castro et al. (1990) reported an average coefficient of variation associated with position in the chamber of 6.8%, considerably higher than the value reported here (1.24%). These results emphasize the importance of the consistency of subject position and restraint technique. In the present study, the prediction equation for lean mass as a function of EM-SCAN number had a considerably lower intercept (−413.93) and higher slope (5.831) than that of Castro et al. (−42.66 and 2.71, respectively). This suggests that a linear regression model is only appropriate over a fairly narrow range of lean body mass and that prediction equations need to be derived for each species to be measured.

Total body electrical conductivity measurements can yield estimates of total body lipid in bobwhites that are within ±10% of actual total body lipid (mean = 10.42%, range = 0.20–64.1%, $n$ = 38). In all respects (precision, subject stress, ease, time required), the TOBEC technique was superior to the IRI technique for nondestructive measurement of total body lipid in bobwhites. The results of the TOBEC validation on bobwhites indicate that this technique will provide new opportunities for measuring body composition over time in individual birds. While the high accuracy of TOBEC body composition analysis makes it an attractive nondestructive method, commercially available TOBEC analyzers currently limit the size range of subjects that can be analyzed. The SA-1 and SA-2 Small Animal Body Composition Analyzer (EM-SCAN, Inc., Springfield, Illinois) can only accommodate birds up to approximately 300 g, and the instrument is apparently most accurate in the range of 50–250 g live mass (Castro et al. 1990, D. D. Roby unpubl. data). Models designed for adult human or pediatric applications are too large for use in the field and are very expensive.

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